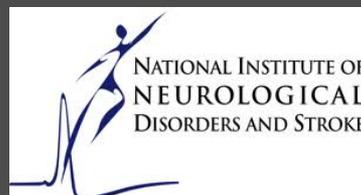




# Optimizing the Predictive Value of Preclinical Research

Ursula Utz  
John Porter  
Amelie Gubitz  
Shai Silberberg

Welcome  
and  
Thanks

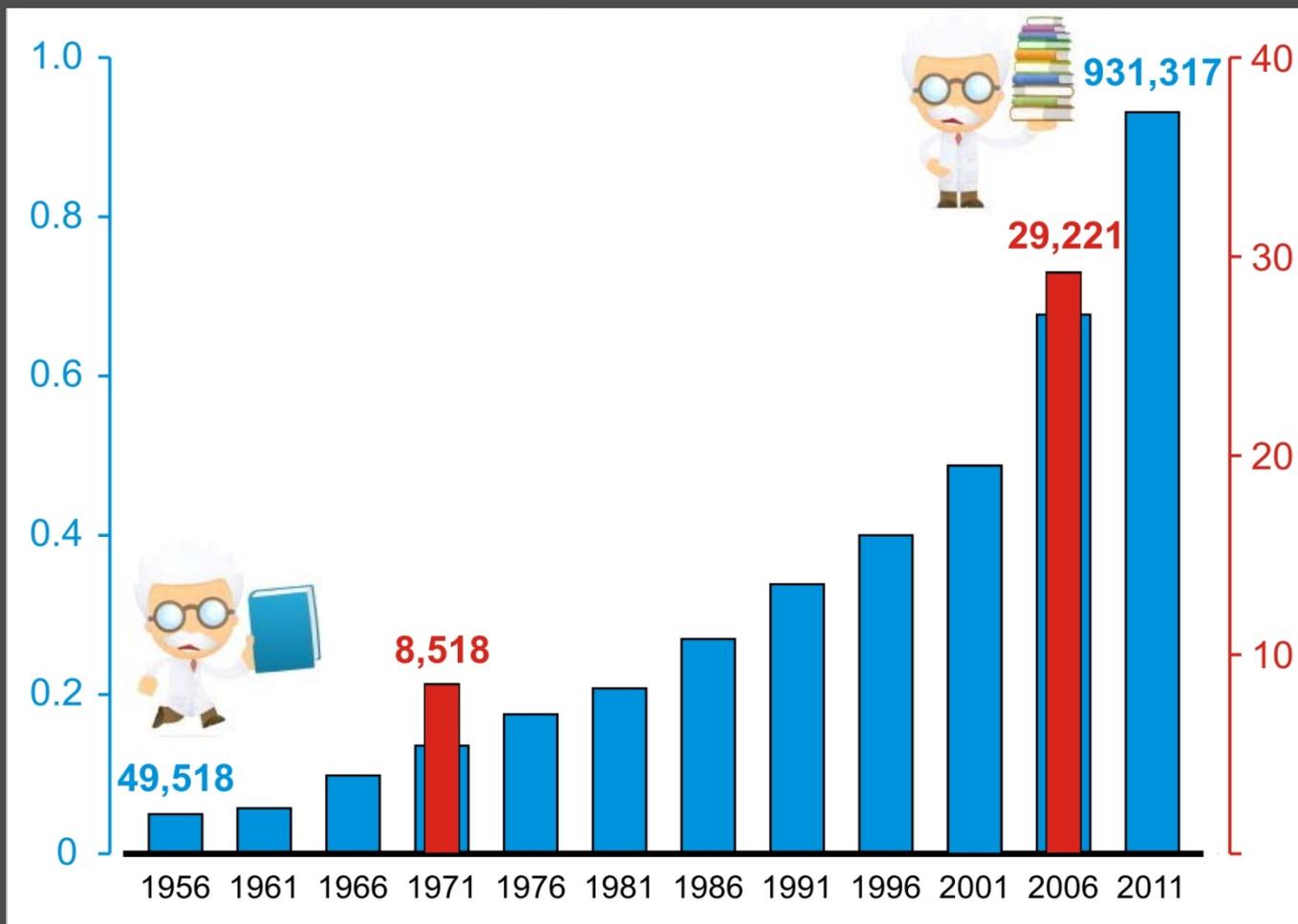


June 20 – 21, 2012  
Washington Plaza Hotel  
Washington DC



# The Escalation in Scientific Reporting (Annual PubMed Publications in English)

Publications  
(x10<sup>6</sup>)



Grants  
(x10<sup>3</sup>)

Year



Editors



Funders



Investigators/  
Readers



Reviewers

# Beware the creeping cracks of bias

*Evidence is mounting that research is riddled with systematic errors. Left unchecked, this could erode public trust, warns Daniel Sarewitz.*

Believe it or not: how much can we rely on published data on potential drug targets?

*Florian Prinz, Thomas Schlange and Khusru Asadullah*

## Statistical Design Considerations in Animal Studies Published Recently in *Cancer Research*

Kenneth R. Hess

## Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

## Why animal research needs to improve

*Many of the studies that use animals to model human diseases are too small and too prone to bias to be trusted, says Malcolm Macleod.*

## False-Positive Psychology: Undisclosed Flexibility in Data Collection and Analysis Allows Presenting Anything as Significant

### Helping editors, peer reviewers and authors improve the clarity, completeness and transparency of reporting health research

David Moher<sup>\*1,2</sup>, Iveta Simera<sup>3</sup>, Kenneth F Schulz<sup>4</sup>, John Hoey<sup>5</sup> and Douglas G Altman<sup>3</sup>

## Reforming Science: Methodological and Cultural Reforms

## Drug targets slip-sliding away

The starting point for many drug discovery programs is a published report on a new drug target. Assessing the reliability of such papers requires a nuanced view of the process of scientific discovery and publication.

### Translating animal research into clinical benefit

Poor methodological standards in animal studies mean that positive results may not translate to the clinical domain



# The definition of bias

“The reliability of a study is determined by the investigator’s choices about critical details of research design and conduct”

(David F. Ransohoff, 2010. *J Clin Oncol* 28: 698-704)

“Bias is unintentional and unconscious. It is defined broadly as the systematic erroneous association of some characteristic with a group in a way that distorts a comparison with another group.....”

“.....The process of addressing bias involves making everything equal during the design, conduct and interpretation of a study, and reporting those steps in an explicit and transparent way.”

# BIAS IN TREATMENT ASSIGNMENT IN CONTROLLED CLINICAL TRIALS

THOMAS C. CHALMERS, M.D., PAUL CELANO, M.D., HENRY S. SACKS, PH.D., M.D.,  
AND HARRY SMITH, JR., PH.D.

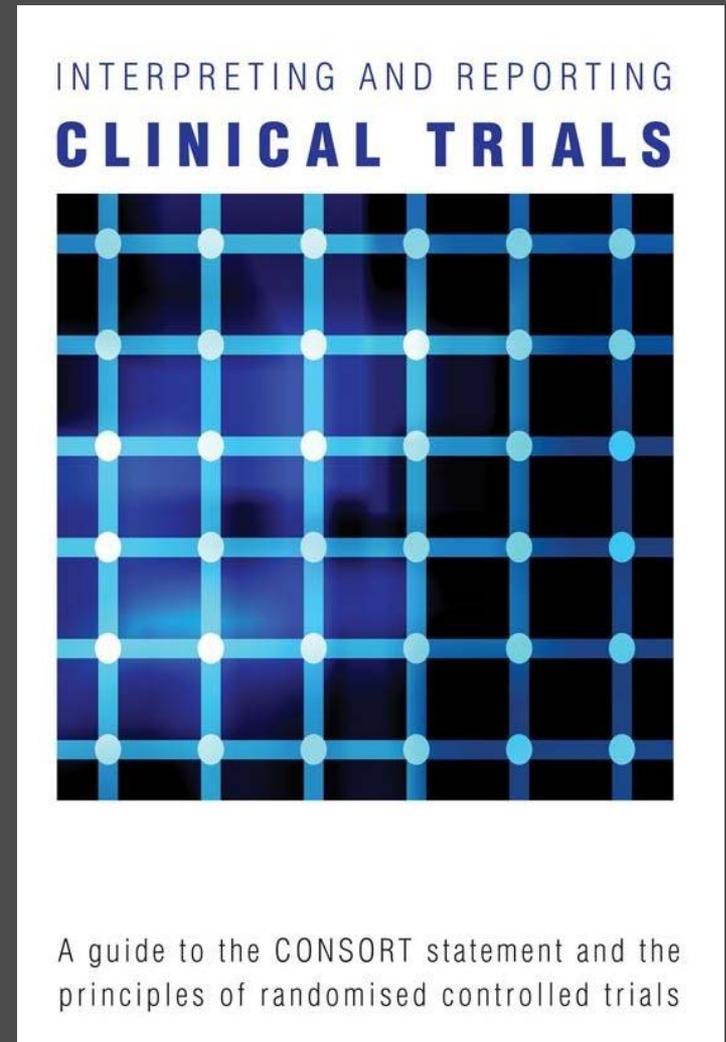
**Table 6. Conclusions of Authors about Efficacy of Treatment (Based on Clinical-Response Data in Addition to Case-Fatality Rate).**

TYPE OF STUDY	TOTAL NO. OF STUDIES	AUTHORS' CONCLUSIONS				
		STRONGLY FAVOR TREATMENT	SLIGHTLY FAVOR TREATMENT	NO PREFERENCE	SLIGHTLY FAVOR CONTROL	STRONGLY FAVOR CONTROL
				<i>per cent</i>		
Blinded randomization	57	29.8	21.1	45.6	3.5	0
Unblinded randomization	45	31.1	15.6	48.9	4.4	0
Nonrandom assignment	43	55.8	16.3	20.9	4.7	2.3

# The CONSORT statement provides guidelines for reporting clinical trials

“Randomized trials can yield biased results if they lack methodological rigour.

To assess a trial accurately, readers of a published report need complete, clear, and **transparent** information on its methodology and findings.”





# The design of randomized clinical trials

Blinding

Randomization

Sample size determination

Inclusion / exclusion criteria

Conflict of interest

# What about pre-clinical studies?



*In vitro*



*In vivo*

# Insufficient reporting of methodological approaches is evident for pre-clinical studies

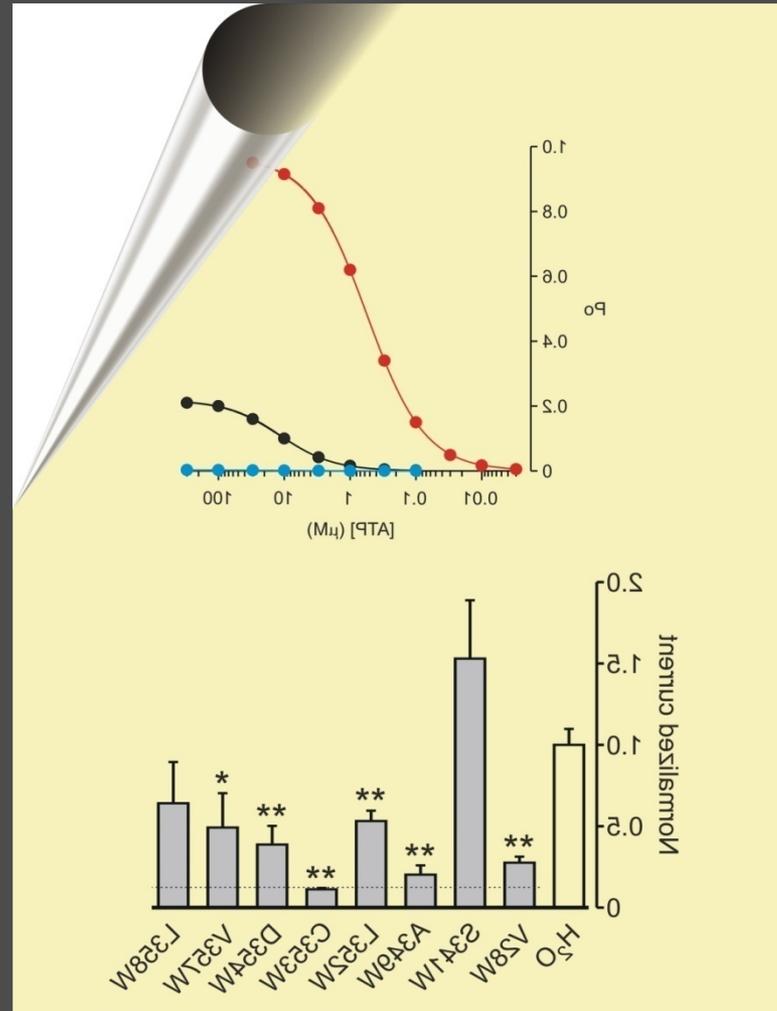
Quality of animal studies. Values are numbers (percentages)

Intervention (No of studies)	Random allocation to group	Adequate allocation concealment	Blinded assessment of outcome
Corticosteroids for traumatic head injury (n=17)	2 (12)	3 (18)	3 (18)
Antifibrinolytic agents (n=8)	3 (38)	0	4 (50)
Thrombolysis for acute ischaemic stroke (n=113)	43 (38)	23 (20)	24 (21)
Tirilazad for acute ischaemic stroke (n=18)	12 (67)	1 (6)	13 (72)
Antenatal corticosteroids (n=56)	14 (25)	0	3 (5)
*Bisphosphonates (n=16)	5 (31)	0	0

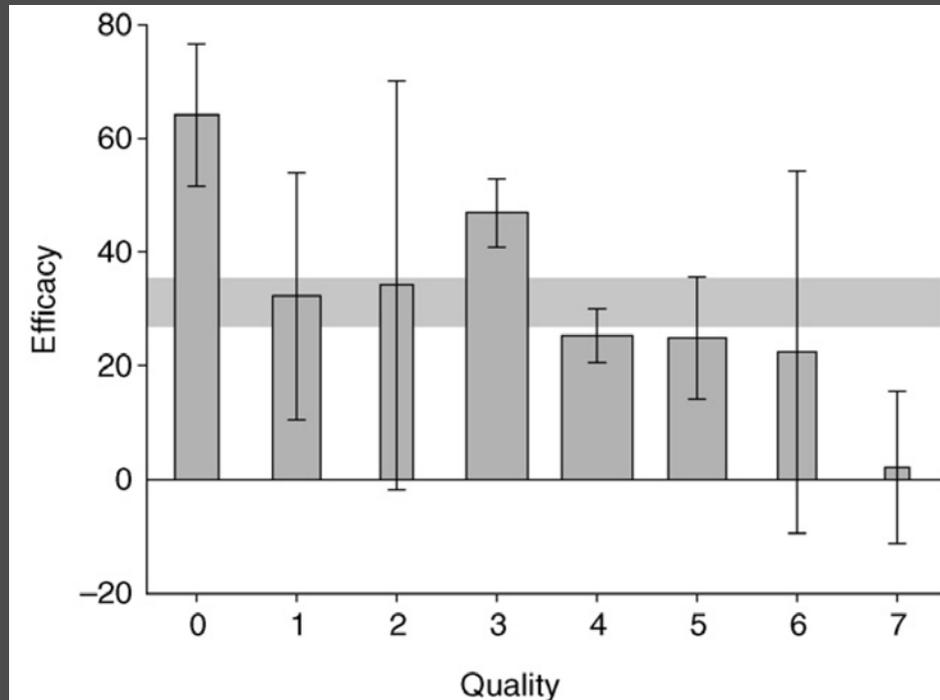
**Table 3. Prevalence of selected quality characteristics in other experimental models**

	Number of publications	Randomisation (%)	Blinded assessment of outcome (%)	Sample-size calculation (%)
Transgenic stroke studies	157	n/a	3	0
Stroke pathophysiology studies	166	5	18	0
Parkinson's disease	118	12	15	0
Multiple sclerosis	183	2	11	0

# Lack of Transparency



# The fewer methodological parameters are reported, the greater the overall effect size!



Effect size for studies of **FK506** (Tacrolimus) in experimental stroke.

# Deficient reporting is widespread

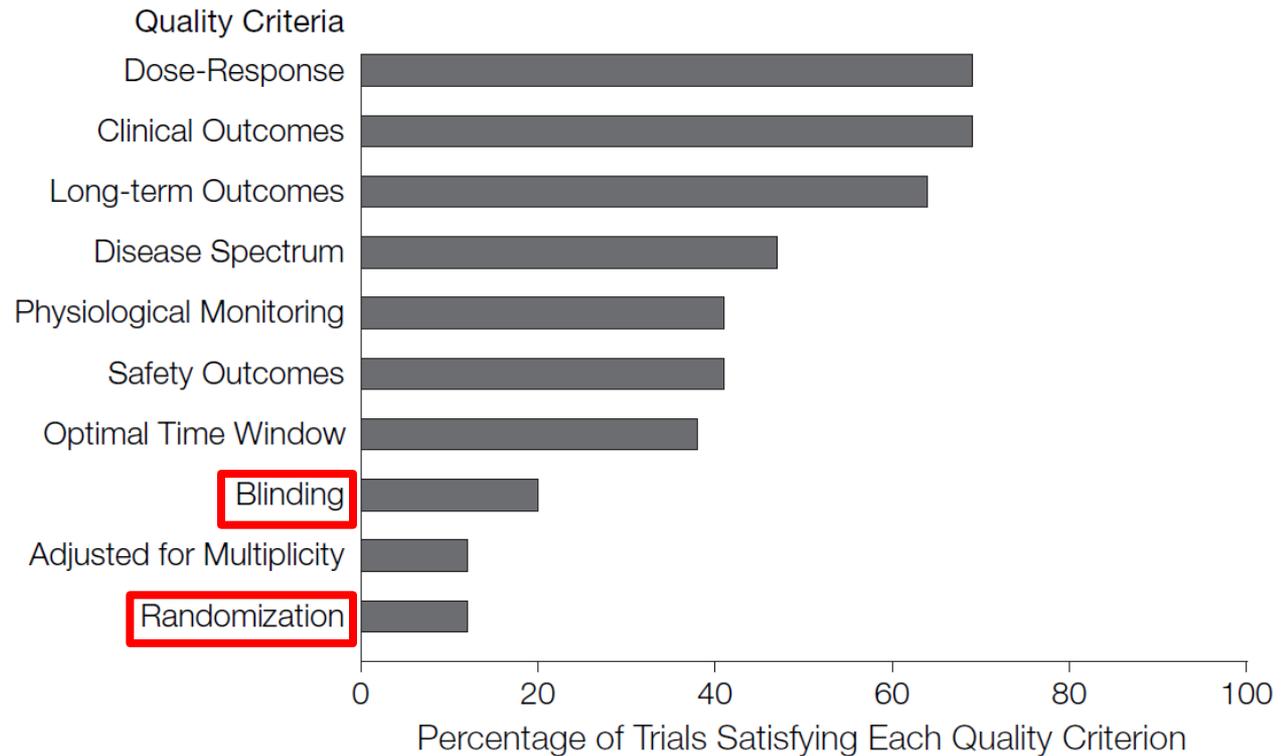
Journals:

- Cell
- Nature
- Science
- Nature Medicine
- Nature Genetics
- Nature Immunology
- Nature Biotechnology

>500 citations

Translated to human studies

**Figure 1.** Methodological Quality of Animal Trials (n=76)



# Chance





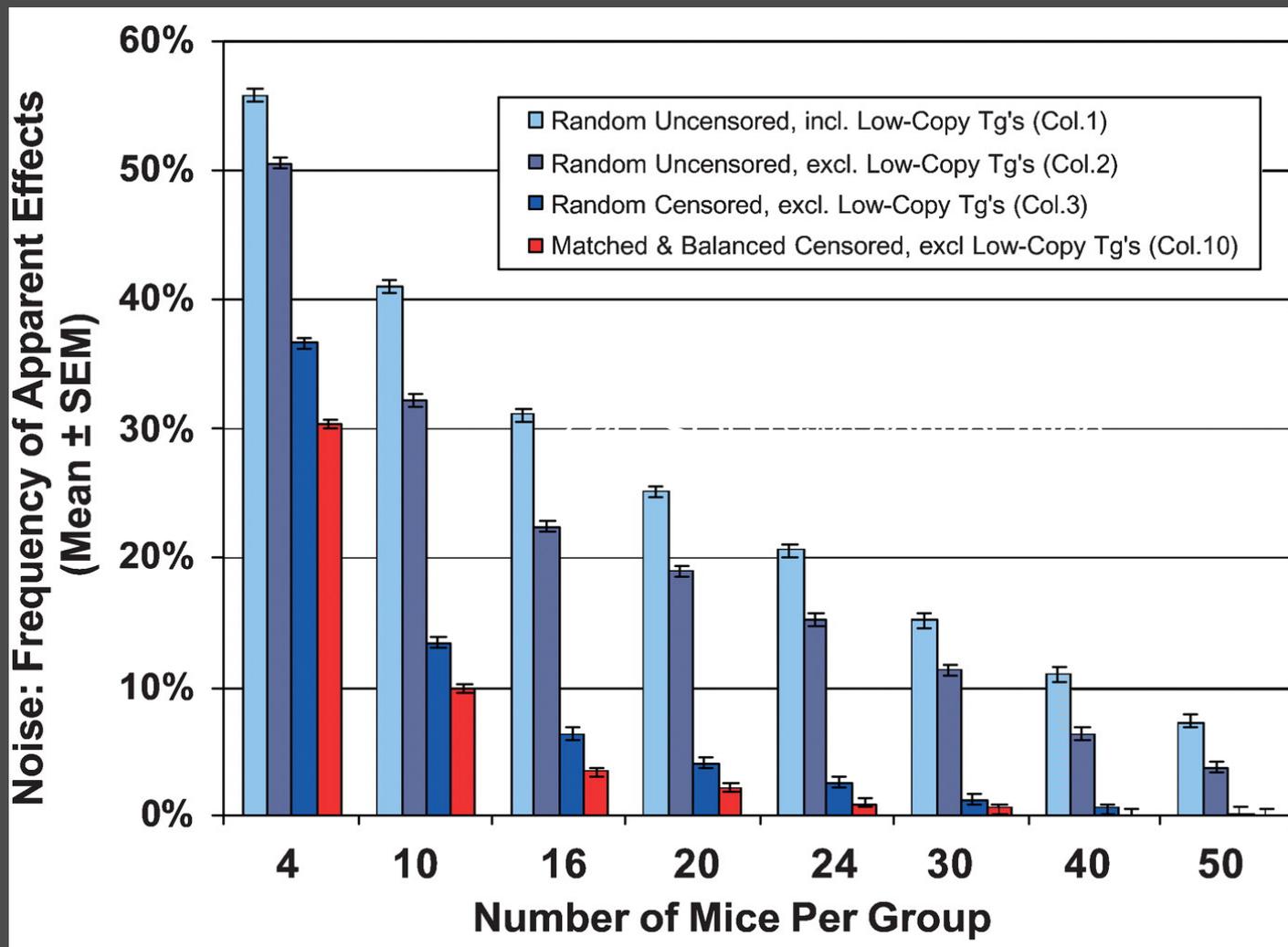
## Design, power, and interpretation of studies in the standard murine model of ALS

SEAN SCOTT<sup>1</sup>, JANICE E. KRANZ<sup>1</sup>, JEFF COLE<sup>1</sup>, JOHN M. LINCECUM<sup>1</sup>,  
KENNETH THOMPSON<sup>1</sup>, NANCY KELLY<sup>1</sup>, ALAN BOSTROM<sup>2</sup>, JILL THEODOSS<sup>1</sup>,  
BASHAR M. AL-NAKHALA<sup>1</sup>, FERNANDO G. VIEIRA<sup>1</sup>, JEYANTHI RAMASUBBU<sup>1</sup> &  
JAMES A. HEYWOOD<sup>1</sup>      **ALS Therapy Development Institute (ALS TDI)**

“In the past five years we have screened more than 70 drugs in 18000 mice across 221 studies, using rigorous and appropriate statistical methodologies. While we were able to measure a significant difference in survival between males and females with great sensitivity, **we observed no statistically significant positive (or negative) effects for any of the 70 compounds tested, including several previously reported as efficacious.** “

“....We retested several compounds reported in major animal studies (minocycline, creatine, celecoxib, sodium phenylbutyrate, ceftriaxone, WHI-P131, thalidomide, and riluzole) ...and **found no survival benefit** in the SOD1(G93A) mouse for any compounds (including riluzole) administered by their previously reported routes and doses. ....the majority of published effects are most likely measurements of noise in the distribution of survival means as opposed to actual drug effect.“

# The probability of seeing an effect by chance alone is significant even with 10 animals per group



# Publication Bias





# Publication decisions and their possible effects on inferences drawn from tests of significance—or vice versa

THEODORE D. STERLING  
University of Cincinnati

“There is some evidence that in fields where statistical tests of significance are commonly used, research which yields nonsignificant results is not published. Such research being unknown to other investigators may be repeated independently until eventually by chance a significant result occurs - an “error of the first kind” - and is published.”

“Significant results published in these fields are seldom verified by independent replication. The possibility thus arises that the literature of such a field consists in substantial part of false conclusions resulting from errors of the first kind in statistical tests of significance.”

*Journal of the American Statistical Association, 1959; 54:30-34*



# “Publication bias in reports of animal stroke studies leads to overstatement of efficacy”

“We identified 16 systematic reviews of interventions tested in animal studies of acute ischaemic stroke involving 525 unique publications.

Only ten publications (2%) reported no significant effects on infarct volume.”

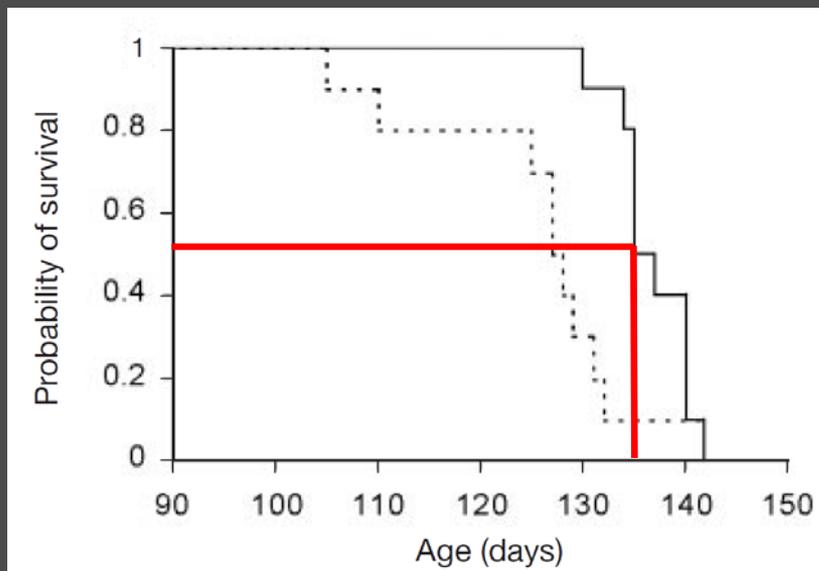
# Almost all articles on cancer prognostic markers report statistically significant results

Panayiotis A. Kyzas<sup>a</sup>, Despina Denaxa-Kyza<sup>a</sup>, John P.A. Ioannidis<sup>a,b,c,\*</sup>

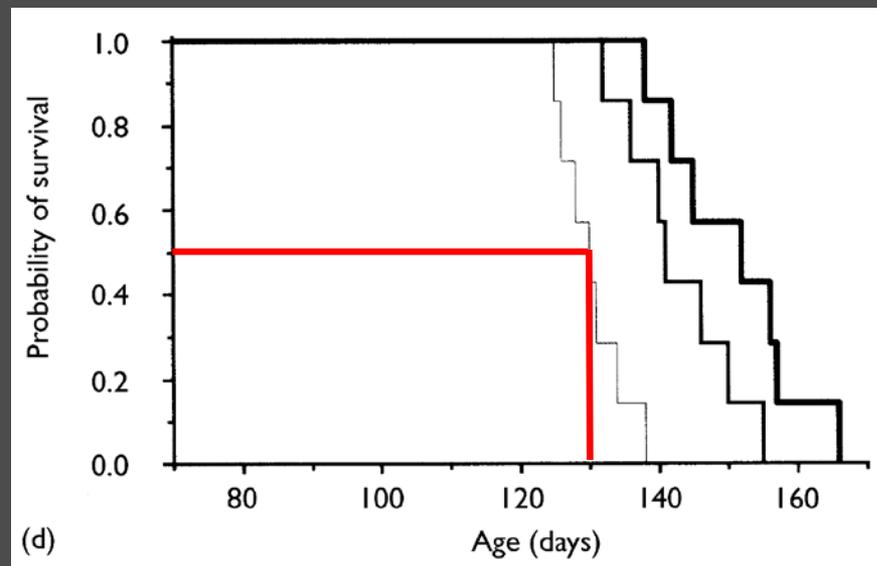
“We evaluated **340** articles included in prognostic marker meta-analyses (Database 1) and **1575** articles on cancer prognostic markers published in 2005 (Database 2).

.....Only five articles in Database 1 (**1.5%**) and 21 in Database 2 (**1.3%**) were fully ‘negative’ for all presented results in the abstract and without efforts to expand on non-significant trends or to defend the importance of the marker with other arguments.”

# The Survival Benefit of Drug X in the SOD1<sup>G93A</sup> Mouse Model of ALS Might be Due to Small Sample Size and/or Bias



- SOD1<sup>G93A</sup> transgenic mice
- Treatment started at 5 weeks of age
- i.p. 10mg/kg/day
- **10** animals / group
- **Not randomized**
- **Not blinded**



- (d) ➤ SOD1<sup>G93A</sup> transgenic mice
- Treatment started at 10 weeks of age
- i.p. 25 and 50 mg/kg/day
- **7** animals / group
- **Not randomized**
- **“The experimenter was blinded to the treatment protocol.”**

## Guidelines for preclinical animal research in ALS/MND: A consensus meeting

ALBERT C. LUDOLPH<sup>1</sup>, CATERINA BENDOTTI<sup>2</sup>, ERAN BLAUGRUND<sup>3</sup>,  
ADRIANO CHIO<sup>4</sup>, LINDA GREENSMITH<sup>5</sup>, JEAN-PHILIPPE LOEFFLER<sup>6</sup>,  
RICHARD MEAD<sup>7</sup>, HEIKO G. NIESSEN<sup>8</sup>, SUSANNE PETRI<sup>9</sup>,  
PIERRE-FRANCOIS PRADAT<sup>10</sup>, WIM ROBBERECHT<sup>11</sup>, MARKUS RUEGG<sup>12</sup>,  
BIRGIT SCHWALENSTÖCKER<sup>1</sup>, DETLEV STILLER<sup>8</sup>, LEONARD VAN DEN BERG<sup>13</sup>,  
FERNANDO VIEIRA<sup>14</sup> & STEPHAN VON HORSTEN<sup>15</sup>

“As an absolute minimum for any study of survival (time to loss of righting reflex), **12 animals of a single sex** must be used unless justified by power analysis with data shown in supplemental figures or tables.”

“Of particular importance is **replication** of the therapeutic effect in an alternative mammalian model or in a different laboratory.”



# Guidelines developed by various disease groups

Stroke: The STAIR preclinical recommendations  
(*Stroke* 2009; 40: 2244-2250)

**Dr. David Howells, Dr. Malcolm Macleod**

ALS: Guidelines for preclinical animal research  
(*ALS* 2010; 11: 38-45)

**Dr. Steve Perrin**

AD: Best practices for preclinical studies  
(Alzheimer's Drug Discovery Foundation and Charles River)

**Dr. Howard Fillit**

HD: Best Practice Guidelines for Therapeutic Target Validation  
(Developed in response to a NINDS RFI)

**Dr. Dimitri Krainc**



Publish or perish!

Grant support

Impact factor



Innovation

Significance

Novelty

# Improve Review of Manuscripts and Grants





# Improving the Quality of NINDS-Supported Preclinical and Clinical Research through Rigorous Study Design and Transparent Reporting

**Notice Number:** NOT-NS-11-023

**Release Date:** August 10, 2011

**Issued by:** National Institute of Neurological Disorders and Stroke (NINDS)

## **Purpose:**

.....NINDS believes that applications that propose preclinical research, or that are based on previous preclinical data, will be greatly strengthened if the design, execution, and interpretation of the proposed studies and supporting data are adequately described. NINDS encourages investigators, whenever possible, to address these elements directly in their applications.



# Points to consider

## Experimental design:

- Rationale for the selected models and endpoints (animal and/or cellular)
- Adequacy of the controls
- Route & timing of intervention delivery / dosing
- Justification of sample size, including power calculation
- Statistical methods used in analysis and interpretation of results

## Minimizing bias:

- Methods of blinding (allocation concealment and blinded assessment of outcome)
- Strategies for randomization and/or stratification
- Reporting of data missing due to attrition or exclusion
- Reporting of all results (negative and positive)

## Results:

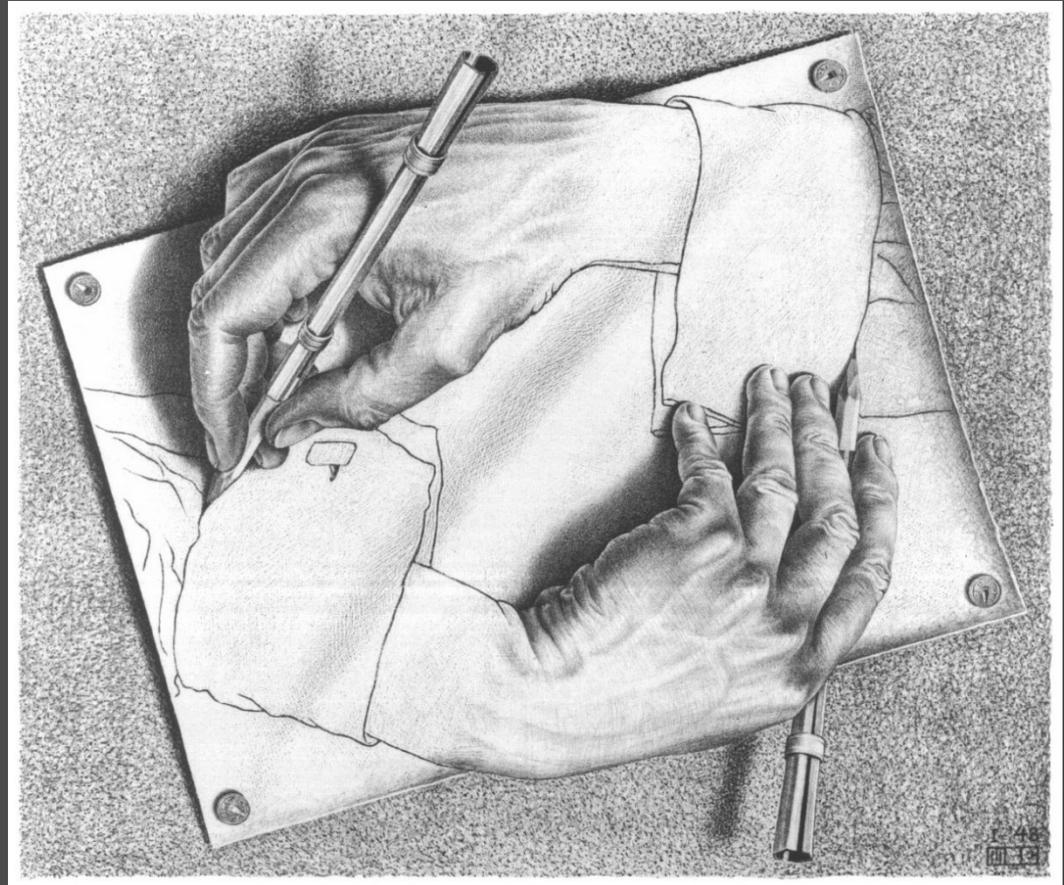
- Independent validation/replication, if available
- Robustness and reproducibility of the observed results
- Dose-response results
- Verification that interventional drug or biologic reached and engaged the target

## Interpretation of results:

- Alternative interpretations of the experimental data
- Relevant literature in support or in disagreement with the results
- Discussion of effect size in relation to potential clinical impact
- Potential conflicts of interest

# Should the Journals play an active role in improving the transparency of reporting?

Are the journals passive conduits of “the best science being done” or do they influence the science being done and its level of reporting?



# Outline of Workshop

## Today:

- 1: Disease community perspective
- 2: Journal perspective
- 3: Reviewer perspective

## Tomorrow:

**Dr. Asadullah: *“Experiences in Collaborating with Academia”***

- 4: Investigator perspective
- 5: **Recommendations for further actions**

# Workshop Goals

- ❖ A consensus short list of items to be reported for *in vivo* animal studies
- ❖ A proposal for how to motivate and incentivize investigators to improve reporting
- ❖ An action plan for improving review
- ❖ A plan for promoting the outcomes of the workshop



# “If There is no Flour There is no Torah”

- ❖ **Coffee** can be purchased in the lobby
- ❖ **Lunch:** Several good simple restaurants are at: **Logan Circle**  
It will be HOT (95° F; 35° C) !!
- ❖ **Dinner:** A list of restaurants is in the folder
- ❖ **Drinks:** Meet at the hotel bar after dinner!

